inhibits cellular responsiveness. B cell activation, B cell proliferation and antibody secretion is thus aborted.

TABLE 1. Receptors for the Fc Regions of Immunoglobulin Isotypes			
Receptor	**Binding**	**Cell Type**	**Effect of Ligation**
FcγRI (CD64)	IgG1 10^8 M^{-1}	Macrophages Neutrophils Eosinophils Dendritic cells	Uptake Stimulation Activation of respiratory burst Induction of killing
FcγRII-A (CD32)	IgG1 2×10^6 M^{-1}	Macrophages Neutrophils Eosinophils Dendritic cells Platelets Langerhan cells	Uptake Granule Release
FcγRII-B2 (CD32)	IgG1 2×10^6 M^{-1}	Macrophages Neutrophils Eosinophils	Uptake Inhibition of Stimulation
FcγRII-B1 (CD32)	IgG1 2×10^6 M^{-1}	B cells Mast cells	No Uptake Inhibition of Stimulation
FcγRIII (CD16)	IgG1 5×10^5 M^{-1}	NK cells Eosinophil Macrophages Neutrophils Mast Cells	Induction of Killing
FcεRI	IgE 10^{10} M^{-1}	Mast cells Eosinophil Basophils	Secretion of granules
FcαRI (CD89)	IgA1, IgA2 10^7 M^{-1}	Macrophages Neutrophils Eosinophils	Uptake Induction of Killing

3. SUMMARY

[0014] The present disclosure relates to covalent diabodies and/or covalent diabody molecules and to their use in the treatment of a variety of diseases and disorders including cancer, autoimmune disorders, allergy disorders and infectious diseases caused by bacteria, fungi or viruses. Preferably, the diabody of the present disclosure can bind to two different epitopes on two different cells wherein the first epitope is expressed on a different cell type than the second epitope, such that the diabody can bring the two cells together.

[0014a] In one aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, said polypeptide chains being covalently bonded to one another, wherein:

I. said first polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1); (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation;

which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

II. said second polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for said epitope (2); (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for said epitope (1),

which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

wherein the first domain and the fifth domain associate to form a binding site (VL1)(VH1) that binds said epitope (1);

wherein the second domain and the fourth domain associate to form a binding site (VL2)(VH2) that binds said epitope (2)

[0015] In one embodiment, the present disclosure is directed to a covalent bispecific diabody, which diabody comprises a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope, and, optionally, (iii) a third domain

chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain are covalently linked, with the proviso that the covalent link is not a peptide bond; wherein the first domain and the fifth domain associate to form a first binding site (VLl)(VHl) that binds the first epitope; wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds the second epitope.

[0018] In certain embodiments, the present disclosure is directed to a covalent bispecific diabody, which diabody is a dimer of diabody molecules, each diabody molecule comprising a first and a second polypeptide chain, which first polypeptide chain comprises (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VLl) specific for a first epitope, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for a second epitope and (iii) a third domain comprising an Fc domain or portion thereof, which first and second domains are covalently linked such that the first and second domains do not associate to form an epitope binding site; and which second polypeptide chain comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2), (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VHl), and (iii) a sixth domain comprising at least one cysteine residue, which fourth and fifth domains are covalently linked such that the fourth and fifth domains do not associate to form an epitope binding site; and wherein the first polypeptide chain and the second polypeptide chain of each diabody molecule are covalently linked, with the

embodiment of the disclosure, at least one epitope binding site of the diabody or diabody molecule is specific for an activating Fc receptor and at least one other site is specific for an inhibitory Fc receptor. In certain embodiments the activating Fc receptor is CD32A and the inhibitory Fc receptor is CD32B. In other embodiments the activating Fc receptor is BCR and the inhibitory Fc receptor is CD32B. In still other embodiments, the activating Fc receptor is IgERI and the inhibitory Fc receptor is CD32B.

[0031a] In another aspect, there is provided a diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, wherein:

said first polypeptide chain comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein said cysteine residue of said third and sixth domains form a disulfide bond between said first and second polypeptide chains;

wherein when said first polypeptide chain comprises said E-coil separator, said second polypeptide chain comprises said K-coil separator; and when said first polypeptide chain comprises said K-coil separator, said second polypeptide chain comprises said E-coil separator;

wherein the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds CD79b.

[0031b] In another aspect, there is provided a diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain;

which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;
said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;
wherein when said first polypeptide chain of said first diabody comprises said E-oil separator, said second polypeptide chain of said first diabody comprises said K-coil separator; and when said first polypeptide chain of said first diabody comprises said K-coil separator, said second polypeptide chain of said first diabody comprises said E-coil separator;
wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;
wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;
wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b; and
(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:
said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain;
which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;
said second polypeptide chain of said second diabody molecule comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;
wherein when said first polypeptide chain of said second diabody comprises said E-coil separator, said second polypeptide chain of said second diabody comprises said K-coil separator; and when said first polypeptide chain of said second diabody comprises said K-coil separator, said second polypeptide chain of said second diabody comprises said E-coil separator;
wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;
wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b;
wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecules to form an Fc region.

[0031c] In another aspect, there is provided a diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:
said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain;
which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;
said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;
wherein when said first polypeptide chain of said first diabody comprises said E-coil separator, said second polypeptide chain of said first diabody comprises said K-coil separator; and when said first polypeptide chain of said first diabody comprises said K-coil separator, said second polypeptide chain of said first diabody comprises said E-coil separator;
wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;
wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;
wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B; and

(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:
said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for

CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said second diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said second diabody comprises said E-coil separator, said second polypeptide chain of said second diabody comprises said K-coil separator; and when said first polypeptide chain of said second diabody comprises said K-coil separator, said second polypeptide chain of said second diabody comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;

wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B;

wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecules to form an Fc region.

[0032] In cases where one epitope binding site is specific for CD16A, the VL and VH domains can be the same as or similar to the VL and VH domains of the mouse antibody 3G8, the sequence of which has been cloned and is set forth herein. In other cases where one epitope binding site is specific for CD32A, the VL and VH domains can be the same as or similar to the VL and VH domains of the mouse antibody IV.3. In yet other cases where one epitope binding site is specific for CD32B, the VL and VH domains can be the same as or similar to the VL and VH domains of the mouse antibody 2B6, the sequence of which has been cloned and is set forth herein. It is to be understood that any of the VL or VH domains of the 3G8, 2B6 and IV.3 antibodies can be used in any combination. The present disclosure is also directed to a bispecific diabody or diabody molecule wherein the first epitope is specific for CD32B, and the second epitope is specific for CD16A.

[0033] In other embodiments, an epitope binding site can be specific for a pathogenic antigen. As used herein, a pathogenic antigen is an antigen involved in a specific pathogenic disease, including cancer, infection and autoimmune disease. Thus, the pathogenic antigen can be a tumor antigen, a bacterial antigen, a viral antigen, or an autoimmune antigen. Exemplary pathogenic antigens include, but are not limited to lipopolysaccharide, viral

antigens selected from the group consisting of viral antigens from human immunodeficiency virus, Adenovirus, Respiratory Syncitial Virus, West Nile Virus (e.g., E16 and/or E53 antigens) and hepatitis virus, nucleic acids (DNA and RNA) and collagen. Preferably, the pathogenic antigen is a neutralizing antigen. In a preferred embodiment, where one epitope binding site is specific for CD16A or CD32A, the other epitope binding site is specific for a pathogenic antigen excluding autoimmune antigens. In yet another preferred embodiment, where one epitope binding site is specific for CD32B, the other epitope binding site is specific for any pathogenic antigen. In specific embodiments, the diabody molecule of the disclosure binds two different antigens on the same cell, for

example, one antigen binding site is specific for an activating Fc receptor while the other is specific for an inhibitory Fc receptor. In other embodiments, the diabody molecule binds two distinct viral neutralizing epitopes, for example, but not limited to, E16 and E53 of West Nile Virus.

[0034] In yet another embodiment of the present disclosure, the diabodies of the disclosure can be used to treat a variety of diseases and disorders. In another aspect, there is provided a pharmaceutical composition comprising the diabody molecule as described herein and a pharmaceutically acceptable carrier. In another aspect, there is provided use of the diabody molecule described herein or the pharmaceutical composition described herein for the manufacture of a medicament for inhibiting B-cell proliferation. In another aspect, there is provided use of the diabody molecule described herein or the pharmaceutical composition described herein for the manufacture of a medicament for inhibiting B-cell activation. In another aspect, there is provided use of the diabody molecule described herein or the pharmaceutical composition described herein for the manufacture of a medicament for the treatment of an autoimmune disorder. Accordingly, the present disclosure is directed to a method for treating a disease or disorder comprising administering to a patient in need thereof an effective amount of a covalent diabody or diabody molecule of the disclosure in which at least one binding site is specific for a pathogenic antigen, such as an antigen expressed on the surface of a cancer cell or on the surface of a bacterium or virion and at least one other binding site is specific for a Fc receptor, e.g., CD16A.

[0035] In yet another embodiment, the disclosure is directed to a method for treating a disease or disorder comprising administering to a patient in need thereof an effective amount of a diabody or diabody molecule of the disclosure, in which at least one binding site is specific for CD32B and at least one other binding site is specific for CD16A.

[0036] In yet another embodiment, the disclosure is directed to a method for inducing immune tolerance to a pathogenic antigen comprising administering to a patient in need there an effective amount of a covalent diabody or dovalent diabody molecule of the disclosure, in which at least one binding site is specific for CD32B and at least one other binding site is specific for said pathogenic antigen. In this embodiment, the pathogenic antigen can be an allergen or another molecule to which immune tolerance is desired, such as a protein expressed on transplanted tissue.

[0037] In yet another embodiment, the present disclosure is directed to a method for detoxification comprising administering to a patient in need thereof an effective amount of a covalent diabody or diabody molecule of the disclosure, in which at least one binding site is specific for a cell surface marker and at least one other binding site is specific for a toxin. In particular embodiments, the diabody of the disclosure administered is one where one binding site is specific for a cell surface marker such as an Fc and the other binding site is specific for a bacterial toxin or for a drug. In one embodiment, the cell surface marker is not found on red blood cells.

WHAT IS CLAIMED IS

Claim 1. A diabody molecule comprising a first polypeptide chain and a second polypeptide chain, said polypeptide chains being covalently bonded to one another, wherein:

I. said first polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1); (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation;

which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

II. said second polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for said epitope (2); (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for said epitope (1),

which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form said epitope (1) binding site or said epitope (2) binding site;

wherein the first domain and the fifth domain associate to form a binding site (VL1)(VH1) that binds said epitope (1);

wherein the second domain and the fourth domain associate to form a binding site (VL2)(VH2) that binds said epitope (2).

Claim 2. The diabody molecule of claim 1, wherein said second polypeptide chain additionally comprises: (iii) a negatively or positively charged polypeptide domain that spontaneously assumes a helical conformation linked to said fifth

domain, wherein one of said first and second polypeptide chains contains said positively charged polypeptide domain that spontaneously assumes a helical conformation and the other of said first and second polypeptide chains contains said negatively charged polypeptide domain that spontaneously assumes a helical conformation.

Claim 3. The diabody molecule of claim 1 or 2, wherein said first polypeptide chain additionally comprises a third domain, said third domain being separated from said second domain by said charged polypeptide domain, wherein said third domain is an Fc domain or portion thereof.

Claim 4. The diabody molecule of any one of claims 1-3, wherein said second polypeptide chain additionally comprises a sixth domain, said sixth domain being separated from said fifth domain by said charged polypeptide domain, wherein said sixth domain is an Fc domain or portion thereof.

Claim 5. The diabody molecule of any one of claims 1-3, wherein said diabody additionally comprises a third polypeptide chain and a fourth polypeptide chain, said third and fourth polypeptide chains being covalently bonded to one another, wherein:

III. said third polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a seventh domain comprising a binding region of a light chain variable domain of a third immunoglobulin (VL3) specific for an epitope (3), (ii) an eighth domain comprising a binding region of a heavy chain variable domain of a fourth immunoglobulin (VH4) specific for an epitope (4), (iii) a positively or negatively charged polypeptide domain that spontaneously assumes a helical conformation, and (iv) a ninth domain, wherein said ninth domain is an Fc domain or portion thereof;

which seventh domain and eighth domain are covalently linked such that the seventh domain and eighth domain do not associate together to form said epitope (3) binding site or said epitope (4) binding site;

IV. said fourth polypeptide chain comprises, in the N-terminal to C-terminal direction: (i) a tenth domain comprising a binding region of a light chain variable domain of the fourth immunoglobulin (VL4) specific for said epitope (4), (ii) an eleventh domain comprising a binding region of a heavy chain variable domain of the third immunoglobulin (VH3) specific for said epitope (3);

which tenth domain and eleventh domain are covalently linked such that the tenth domain and eleventh domain do not associate together to form said epitope (3) binding site or said epitope (4) binding site;

wherein the seventh domain and the eleventh domain associate together to form a binding site (VL3)(VH3) that binds said epitope (3);

wherein the eighth domain and the tenth domain associate together to form a binding site (VL4)(VH4) that binds said epitope (4).

Claim 6. The diabody molecule of claim 5, wherein said fourth polypeptide chain additionally comprises: (iii) a negatively or positively charged polypeptide domain that spontaneously assumes a helical conformation linked to said eleventh domain, wherein one of said third and fourth polypeptide chains contains said positively charged polypeptide domain that spontaneously assumes a helical conformation and the other of said third and fourth polypeptide chains contains said negatively charged polypeptide domain that spontaneously assumes a helical conformation.

Claim 7. The diabody molecule of any one of claims 1-6, wherein said negatively charged polypeptide domain that spontaneously assumes a helical conformation is an E-coil **(SEQ ID NO:299)**.

Claim 8. The diabody molecule of any one of claims 1-7, wherein said positively charged polypeptide domain that spontaneously assumes a helical conformation is a K-coil **(SEQ ID NO:300)**.

Claim 9. The diabody molecule of claim 1 or 2, wherein said first polypeptide chain additionally comprises a polypeptide portion of a protein that binds to a serum protein, said polypeptide portion being capable of binding to said serum protein.

Claim 10. The diabody molecule of claim 9, wherein said second polypeptide chain additionally comprises a polypeptide portion of a protein that binds to a serum protein, said polypeptide portion being capable of binding to said serum protein.

Claim 11. The diabody molecule of claim 9 or 10, wherein said protein that binds to a serum protein is an albumin binding protein.

Claim 12. The diabody molecule of claim 11, wherein said albumin binding protein is streptococcal protein G and said polypeptide portion is an albumin-binding domain (ABD) of said streptococcal protein G.

Claim 13. The diabody molecule of claim 12, wherein said albumin-binding domain (ABD) of said streptococcal protein G is albumin-binding domain 3 (ABD3) of protein G of Streptococcus strain G148.

Claim 14. The diabody molecule of any one of claims 9-13, wherein said diabody molecule exhibits an in vivo serum half-life of greater than 2 hours.

Claim 15. The diabody molecule of claim 14, wherein said diabody molecule exhibits an in vivo serum half-life of greater than 10 hours.

Claim 16. The diabody molecule of claim 15, wherein said diabody exhibits an in vivo serum half-life greater than 20 hours.

Claim 17. The diabody molecule of any one of claims 1-15, having a domain that binds an epitope of CD32B and a domain that binds an epitope of CD16.

Claim 18. The diabody molecule of any of claims 1-15, having a domain that is a binding ligand for the Natural Killer Group 2D (NKG2D) receptor.

Claim 19. The diabody molecule of any one of claims 1-15, having a domain that is a binding ligand for the T-cell receptor (TCR) binding domain.

Claim 20. The diabody molecule of any one of claims 1-19, wherein said molecule binds to a tumor-associated antigen.

Claim 21. The diabody molecule of claim 20, wherein said tumor-associated antigen is a breast cancer antigen, an ovarian cancer antigen, a prostate cancer antigen, a cervical cancer antigen, a pancreatic carcinoma antigen, a lung cancer antigen, a bladder cancer antigen, a colon cancer antigen, a testicular cancer antigen, a glioblastoma cancer antigen, an antigen associated with a B cell malignancy, an antigen associated with multiple myeloma, an antigen associated with non-Hodgkin's lymphoma, or an antigen associated with chronic lymphocytic leukemia.

Claim 22. The diabody molecule of claim 20, wherein said tumor-associated antigen is A33; ADAM-9; ALCAM; B1; BAGE; beta-catenin; CA125; Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD32B; CD36; CD40; CD45; CD46; CD56; CD79a; CD79b; CD103; CD154; CDK4; CEA; CTLA4; Cytokeratin 8; EGF-R; an Ephrin receptor; ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2; GD3; GM2; gp100; HER-2/neu; human papillomavirus-E6; human papillomavirus-E7; Integrin Alpha-V-Beta-6; JAM-3; KID3; KID31; KSA; LUCA-2; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; N-acetylglucosaminyltransferase; Oncostatin M; p15; PlPA; PSA; PSMA; RAAG10; ROR1; SART; sTn; TES7; the TNF-α receptor; the TNF-ß receptor; the TNF-γ receptor; the Transferrin Receptor or the VEGF receptor.

Claim 23. The diabody molecule of claim 22, wherein said tumor-associated antigen is HER-2/neu.

Claim 24. A diabody molecule comprising a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second

polypeptide chain are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, wherein:

said first polypeptide chain comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein said cysteine residue of said third and sixth domains form a disulfide bond between said first and second polypeptide chains;

wherein when said first polypeptide chain comprises said E-coil separator, said second polypeptide chain comprises said K-coil separator; and when said first polypeptide chain comprises said K-coil separator, said second polypeptide chain comprises said E-coil separator;

wherein the first domain and the fifth domain associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain associate to form a second binding site (VL2)(VH2) that binds CD79b.

Claim 25. The diabody molecule of claim 24, wherein said first polypeptide chain or said second polypeptide chain further comprises an Fc domain or portion thereof.

Claim 26. The diabody molecule of claim 25, wherein said Fc domain or portion thereof is linked to said E-coil separator or said K-coil separator.

Claim 27. The diabody molecule of claim 25, wherein said Fc domain or portion thereof is linked to the N-terminus of said first domain or said fourth domain.

Claim 28. A diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said first diabody comprises said E-oil separator, said second polypeptide chain of said first diabody comprises said K-coil separator; and when said first polypeptide chain of said first diabody

comprises said K-coil separator, said second polypeptide chain of said first diabody comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;

wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b; and

(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD32B, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD79b, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said second diabody molecule comprises (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD79b, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD32B, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are

covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said second diabody comprises said E-coil separator, said second polypeptide chain of said second diabody comprises said K-coil separator; and when said first polypeptide chain of said second diabody comprises said K-coil separator, said second polypeptide chain of said second diabody comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD32B;

wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD79b;

wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecules to form an Fc region.

Claim 29. A diabody that is a dimer of two diabody molecules, wherein:

(A) the first of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said first diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said first diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD32B, and (iii) a third domain comprising at least one cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said first diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said first diabody comprises said E-coil separator, said second polypeptide chain of said first diabody comprises said K-coil separator; and when said first polypeptide chain of said first diabody comprises said K-coil separator, said second polypeptide chain of said first diabody comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said first diabody molecule form a disulfide bond between said first and second polypeptide chains of said first diabody molecule;

wherein the first domain and the fifth domain of said first diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;

wherein the second domain and the fourth domain of said first diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B; and

(B) the second of said two diabody molecules comprises a first polypeptide chain and a second polypeptide chain, wherein said first polypeptide chain and said second polypeptide chain of said second diabody molecule are covalently linked to one another, with the proviso that the covalent link is not a peptide bond, and wherein:

said first polypeptide chain of said second diabody molecule comprises: (i) a first domain comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for CD79b, (ii) a second domain comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for CD32B, and (iii) a third domain comprising at least one

cysteine residue, an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), and a CH2 domain and a CH3 domain; which first domain and second domain are covalently linked such that the first domain and second domain do not associate to form a first epitope binding site;

said second polypeptide chain of said second diabody molecule comprises: (i) a fourth domain comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for CD32B, (ii) a fifth domain comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for CD79b, and (iii) a sixth domain comprising at least one cysteine residue, and an E-coil separator (SEQ ID NO:299) or a K-coil separator (SEQ ID NO:300), which fourth domain and fifth domain are covalently linked such that the fourth domain and fifth domain do not associate to form an epitope binding site;

wherein when said first polypeptide chain of said second diabody comprises said E-coil separator, said second polypeptide chain of said second diabody comprises said K-coil separator; and when said first polypeptide chain of said second diabody comprises said K-coil separator, said second polypeptide chain of said second diabody comprises said E-coil separator;

wherein said cysteine residue of said third and sixth domains of said second diabody molecule form a disulfide bond between said first and second polypeptide chains of said second diabody molecule;

wherein the first domain and the fifth domain of said second diabody molecule associate to form a first binding site (VL1)(VH1) that binds CD79b;

wherein the second domain and the fourth domain of said second diabody molecule associate to form a second binding site (VL2)(VH2) that binds CD32B;

wherein said CH2 domain and CH3 domain of said first polypeptide chain of said first diabody associates with said CH2 domain and CH3 domain of said first polypeptide chain of said second diabody molecules to form an Fc region.

Claim 30. The diabody molecule of claim 24, wherein both said E-coil separator and said K-coil separator are linked to an Fc domain or portion thereof.

Claim 31. The diabody molecule of claim 24, wherein said E-coil separator or said K-coil separator is linked to a polypeptide portion of a serum-binding protein, said polypeptide portion being capable of binding to said serum-binding protein.

Claim 32. The diabody molecule of claim 31, wherein said serum-binding protein is an albumin binding protein.

Claim 33. The diabody molecule of claim 32, wherein said albumin binding protein is streptococcal protein G and said polypeptide portion is an albumin-binding domain (ABD) of said streptococcal protein G.

Claim 34. The diabody molecule of claim 33, wherein said albumin-binding domain (ABD) of said streptococcal protein G is albumin-binding domain 3 (ABD3) of protein G of Streptococcus strain G148.

Claim 35. The diabody molecule of claim 31, wherein said diabody molecule exhibits an in vivo serum half-life greater than 2 hours.

Claim 36. The diabody molecule of claim 35, wherein said diabody exhibits an in vivo serum half-life greater than 10 hours.

Claim 37. The diabody molecule of claim 36, wherein said diabody exhibits an in vivo serum half-life greater than 20 hours.

Claim 38. The diabody molecule of any one of claims 24-28 and 30-37, wherein:

(A) said first polypeptide chain comprises the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:252; and

(B) said second polypeptide chain comprises:

(i) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:254; or

(ii) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:256.

Claim 39. The diabody molecule of claim 38, wherein said second polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:254.

Claim 40. The diabody molecule of claim 38, wherein said second polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:256.

Claim 41. The diabody molecule of claim 29, wherein:

(A) said first polypeptide chain comprises:

(i) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:254; or

(ii) the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:256; and

(B) said second polypeptide chain comprises the light chain variable domain, the intervening GGGSGGGG linker (SEQ ID NO:10) and the heavy chain variable domain of SEQ ID NO:252.

Claim 42. The diabody molecule of claim 41, wherein said first polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:254.

Claim 43. The diabody molecule of claim 42, wherein said first polypeptide chain comprises said light chain variable domain, said intervening GGGSGGGG linker (SEQ ID NO:10) and said heavy chain variable domain of SEQ ID NO:256.

Claim 44. A pharmaceutical composition comprising the diabody molecule of any one of claims 24-43 and a pharmaceutically acceptable carrier.

Claim 45. Use of the diabody molecule of any one of claims 24-43 or the pharmaceutical composition of claim 44 for the manufacture of a medicament for inhibiting B-cell proliferation.

Claim 46. Use of the diabody molecule of any one of claims 24-43 or the pharmaceutical composition of claim 44 for the manufacture of a medicament for inhibiting B-cell activation.

Claim 47. Use of the diabody molecule of any one of claims 24-43 or the pharmaceutical composition of claim 44 for the manufacture of a medicament for the treatment of an autoimmune disorder.